Please add the following new claim:

--11. A whole blood immunoassay comprising the steps of:

mixing a whole blood sample, which comprises an antigen and an antibody, with immuno-sensitized insoluble carrier particles to cause an immune agglutination prior to adding a lysing agent;

diluting the resulting agglutination mixture with an aqueous solution containing an erythrocyte lysing agent to lyse erythrocytes; and

determining a degree of agglutination of resulting whole blood sample.--

**REMARKS**

**Priority**

The Examiner is respectfully requested in the next Official Communication to check-off box 13 in the Office Action Summary in accordance with standard USPTO practice. The Examiner's actions and comments are not entirely correct. There is no requirement for Applicants to file a certified English translation of the priority document in order for the Examiner to acknowledge Applicants' properly requested claim for priority.

Moreover, the Examiner improperly checked off item number 14 in the Office Action Summary under the section "Priority under 35 U.S.C. 119 and 120", that is, acknowledgement of domestic priority under 35 U.S.C. 119(e) to a provisional application.

Accordingly, as stated above, the Examiner should properly check item no. 13 including 13a) "All" and 13a)1. "Certified copies of the priority documents have been received." This action will properly acknowledge Applicants' claim for foreign priority. In support of Applicants' position, attached please find a copy of the letter claiming priority as filed on September 27, 2001. Applicants decline the Examiner's invitation to incur the expense of providing an unnecessary certified English translation of the priority document at this time.

**Information Disclosure Statement (IDS)**

An IDS was filed on July 27, 2001 (paper no. 2). The Examiner indicates that she has not considered JP-B 22-912413 because it does not contain a concise explanation of the relevant portions that cause it to be cited. In order to clarify this issue, the Examiner should note that JP-B 22-912413 corresponds to Japanese Unexamined Patent Publication No. HEI 2(1990)-81749, which corresponds to U.S. Patent 5,527,714 to Kosako. Thus, the cited disclosure, which is substantially the

same, is cumulative to the Kosako reference, which is already of record. An abstract of JP 2(1990)-81749 is attached hereto.

**Specification**

The specification has been amended to correct minor typographical or idiomatic errors. Further, the term referred to by the Examiner on pages 8 and 12 of the specification has been capitalized. No new matter has been added.

The first sentence of the specification properly recites Applicants claim for priority under 35 U.S.C. 119. Applicants also incorporate the priority document by reference. This practice is hardly improper. See MPEP 608.01(p) I.B at page 600-80 and 600-81 (August 2001) which supports Applicants' position.

**Claim Rejections - 35 USC 112**

Claims 1-10 (specifically claims 1 and 5) are rejected by the Examiner under 35 U.S.C. 112, second paragraph, for the reasons set forth in paragraph 8 of the Office Action. This rejection is respectfully traversed. Reconsideration and withdrawal thereof are requested.

The Examiner's position with respect to claim 1 is that the last step of the claim is not consistent with the preamble of the claim, and that it is not clear as to what is being agglutinated. Claim 1 has been amended to merely clarify the

invention. No new matter or narrowing amendments have been introduced.

More specifically, the degree of agglutination is that of the claimed carrier particles with that of an antibody or an antigen within the claimed whole blood sample. According to claim 1, the whole blood sample is mixed with the sensitized (i.e. immuno-sensitized) carrier particles to agglutinate the carrier particles with the antibody or antigen in the whole blood sample. In the present invention, the erythrocyte lysing agent is added to the resulting whole blood sample to lyse erythrocytes in the whole blood sample after the agglutination step takes place. The resulting material is the assay sample which is analyzed or assayed for its degree of agglutination.

Accordingly, the assay sample is the whole blood sample itself in which the antibody or antigen is agglutinated with the carrier particles. The erythroblasts are subsequently lysed. Claim 1 has been amended consistent with this explanation.

The Examiner's reference to claim 5 appears to be incorrect. The Examiner appears to intend to refer to claim 4. Claim 4 is amended to recite a whole blood immunoassay wherein the degree of agglutination of the assay sample is conducted (e.g. determined) by flow cytometry. This amendment is for clarification purposes only and does not narrow the scope of the invention.

In view of the amendments to claims 1 and 4 and the remarks hereinabove, reconsideration and withdrawal of the rejections of claims 1-10 under 35 U.S.C. 112, second paragraph, are respectfully requested.

**Claim Rejections - 35 U.S.C. 102**

Claim 1 is rejected by the Examiner under 35 U.S.C. 102(b) over U.S. Patent 6,030,845 to Yamao et al. for the reasons set forth in paragraph 9 of the Office Action. This rejection is respectfully traversed. Reconsideration and withdrawal thereof are requested.

**The Present Invention**

The present invention as recited in claim 1, as amended, relates to a whole blood immunoassay comprising the steps of mixing a whole blood sample with sensitized insoluble carrier particles to cause an immune agglutination, diluting the resulting agglutination mixture with an aqueous solution containing an erythrocyte lysing agent to lyse erythrocytes, and determining a degree of agglutination of resulting whole blood sample.

**U.S. Patent 6,030,845 to Yamao t al.**

The Yamao et al. patent claims priority on JP 8-217966 filed July 30, 1996. The JP 8-217966 application was published as Japanese Unexamined Patent Publication No. HEI 10(1998)-48214.

The Yamao et al. patent discloses the use of whole blood, the use of insoluble particles with an antigen or an antibody immobilized thereon, subjecting the mixture to an agglutination reaction after hemolysis and measuring the agglutinated matter with scattered light.

**Distinctions Between the Present Invention and the Yamao et al. Patent**

The Yamao et al. reference teaches hemolysis with a surfactant prior to agglutination. The present invention overcomes the prior art problems associated with the use of a surfactant when agglutination follows hemolysis. In the present invention, hemolysis is carried out after the agglutination reaction. This is directly opposite to the teachings of the Yamao et al. reference.

The Yamao et al patent only describes that a whole blood sample is first hemolysed before agglutination and that the hemolysed sample is reacted with a CRP antigen-sensitized latex.

The Yamao et al. patent does not disclose (or even suggest) a reaction of an untreated (non-hemolysed) whole blood sample with the CRP antigen-sensitized latex. The Examiner's attention is directed to the following portions of the Yamao et al. reference in support of Applicants' position: Example 1 at col. 4, lines 3-15; Example 2 at col. 5, lines 5-10; Example 3, col. 5, lines 26-37; and Example 4, col. 5, lines 55-61.

The Examiner's attention is directed to a discussion of Japanese Unexamined Patent Publication No. HEI 10(1998)-48214 corresponding to the Yamao et al. reference at (i) page 2, line 20 through page 3, line 4, and (ii) page 7, line 8 through page 8, line 1 of the present specification. The description at page 2, lines 20 - page 3, line 4 (as amended) is reproduced below for the Examiner's convenience:

> In view of this, for example, Japanese Unexamined Patent Publication No. HEI 10(1998)-48214 discloses a whole blood assay using a conventional latex agglutination method. According to this disclosure, a whole blood sample is hemolyzed with a surfactant and the resulting sample is tested by a latex turbidimetric immunoassay.
>
> However, this assay has a problem in that the surfactant, which needs to be used in a sufficient concentration for hemolysis, affects the antigen-antibody reaction and a sufficient response cannot be obtained.

Accordingly, the Yamao et al. reference teaches hemolysis with a surfactant prior to agglutination. The present invention overcomes the prior art problems associated with the use of a

surfactant when agglutination follows hemolysis. In the present invention, hemolysis is carried out after the agglutination reaction. This is directly opposite to the teachings of the Yamao et al. reference.

The description at page 7, line 8 - page 8, line 1 is reproduced below for the Examiner's convenience:

> In the present invention, after the immune agglutination, erythrocytes are lysed for avoiding their interference with determination before measurement. In an assay as disclosed by Japanese Unexamined Patent Publication No. HEI 10(1998)-48214 in which antigen/antibody reaction is performed after the lysis of erythrocytes, a large amount of a surfactant is required for lysing erythrocytes. In the presence of the surfactant in a large amount, the antigen/antibody reaction is influenced. In order to decrease the concentration of the surfactant used, the whole blood sample needs to be reduced or diluted, which in turn decreases the concentration of an antigen or antibody to be involved in the antigen/antibody reaction and results in a poor response.
> However, if the antigen/antibody reaction is firstly performed under the above-mentioned condition, the antigen/antibody reaction itself is not only affected by the surfactant but also proceeds necessarily and sufficiently. Furthermore, it is possible to detect particles without destroying an antigen/antibody reaction composite (agglutination mixture).

clearly read on any and all lysing agents

Accordingly, when the antigen-antibody agglutination reaction is carried out after hemolysis as in the method of the Yamao et al. reference, a reagent (i.e. surfactant) used for the hemolysis adversely affects the subsequent antigen-antibody reaction. Therefore, sufficient sensitivity cannot be observed

in the Yamao et al. measurement system and a precise measurement cannot be obtained in the Yamao et al. system.

precise sensitivity NOT IN CLAIMS

Thus, the Yamao et al. reference does not destroy the novelty of the present invention since there is no teaching of agglutination followed by hemolysis as in steps 1 and 2 of the present invention.

In order to further understand the differences between the present invention and the cited reference, the Examiner's attention is directed to the side-by-side comparison shown in the Example of the specification. Although a showing of unexpected results cannot be used to overcome a novelty rejection, the side-by-side comparison is useful to show that the Examiner has inadvertently mischaracterized the teachings of the Yamao et al. reference. The Example in the specification clearly demonstrates the differences between the claimed method and the method of the Yamao et al. reference. where Table 1 (limited to SDS measurements)

The Example in the present invention shows the measurement problems referred to in the arguments above with respect to the method of the Yamao et al. reference. Note the various measurements with the serum (control), the agglutination after hemolysis (Yamao et al.) and agglutination followed by hemolysis (the present invention). It is readily evident that the degree of agglutination is significantly different between the

agglutination before hemolysis and after hemolysis as shown in Table 1 of the present specification.

In view of the arguments above, the present invention is clearly not taught by the cited Yamao et al. reference. Thus, the novelty rejection of claim 1 over the Yamao et al reference should be reconsidered and withdrawn by the Examiner.

**Claim Rejections - 35 U.S.C. 103**

Claims 2 and 3 are rejected by the Examiner under 35 U.S.C. 103(a) over U.S. Patent 6,030,845 to Yamao et al. in view of Bester et al. (Analytical Biochemistry, Vol. 223, no. 2, pages 299-305, 1994). The Examiner concedes that Yamao et al. does not disclose the use of an erythrocyte-lysing agent such as sodium dodecyl sulfate to lyse erythrocytes. The Examiner relies on the Bester et al. reference to remedy this deficiency of the Yamao et al. reference.

Claims 4-9 are rejected by the Examiner under 35 U.S.C. 103(a) over U.S. Patent 6,030,845 to Yamao et al. in view of U.S. Patent 5,527,714 to Kosako and U.S. Patent 4,851,329 to Cohen. The Examiner concedes that Yamao et al. does not disclose flow cytometry analysis, particle size and particle to sample ratios. The Examiner relies on the Kosako reference to remedy this deficiency of the Yamao et al. reference. The Examiner alleges that Kosako discloses a method for determining particle

size distributions with respect to an analyte via mediated particle agglutination. See col. 1, lines 30-34 and 65 through col. 2, line 16 and col. 4, lines 36-40 of the Kosako patent and the last paragraph on page 7 through the first paragraph on page 9 of the Office Action. The Examiner states that Cohen et al. discloses a method of determining the concentration of antibody and antigen molecules with high specificity, accuracy and sensitivity. See col. 2, lines 62-67, col. 4, lines 24-26, col. 6, line 57 through col. 7, line 55 of the Cohen et al. patent.

Claim 10 is rejected by the Examiner under 35 U.S.C. 103(a) over U.S. Patent 6,030,845 to Yamao et al. in view of U.S. Patent 4,830,969 to Holmes. The Examiner concedes that Yamao et al. does not disclose immune agglutination reaction temperature and time. The Examiner relies on the Holmes reference to remedy this deficiency of the Yamao et al. reference. The Examiner alleges that Holmes discloses a method for separation of cellular materials. See abstract, col. 1, lines 51-65 and col. 2, lines 1-9 and 54-63 of the Holmes patent and the last two paragraphs on page 9 of the Office Action.

Each of the above-mentioned rejections must fail since the rejection of claim 1 is overcome for the reasons discussed above with respect to the novelty rejection, and the remaining rejections over the dependent claims should be overcome for the same reasons.

More specifically, the primary Yamao et al. reference does not disclose or suggest that hemolysis is carried out after the agglutination reaction. Indeed, modifying the teachings of the Yamao et al. reference in order to obtain the present invention would destroy the teachings of the Yamao et al. reference. Such impermissible hindsight is not allowed. See In re Gordon, 221 USPQ 1125 (Fed. Cir. 1984) and pages 2100-124, 125, MPEP 2143.01 (August 2001).

Moreover, in view of the teachings of the Yamao et al. reference, the combination of the teachings of the Yamao et al. reference with the teachings of any of the secondary references would not lead to the present invention since none of the references disclose or suggest hemolysis conducted after the agglutination reaction. As a result of the present invention, the present inventors have obtained a novel and nonobvious method for providing highly accurate measurements. See the Example in the present specification, as discussed above.

In view of the remarks hereinabove, reconsideration and withdrawal of the various rejections of the claims under 35 U.S.C. 103 are respectfully requested.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

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## Marked-Up Version of the Claims

IN THE SPECIFICATION

The paragraph beginning on page 2, line 20 and ending on line 25, has been amended as follows:

In view of this, for example, Japanese Unexamined Patent Publication No. HEI 10(1998)-48214 discloses a whole blood assay using a conventional latex agglutination method. According to this disclosure, a whole blood sample is hemolyzed [using] with a surfactant and the resulting sample is tested by a latex turbidimetric immunoassay.

The paragraph beginning on page 3, line 1 and ending on line 4, has been amended as follows:

However, this assay has a problem in that the surfactant, which needs to be used in a sufficient concentration for hemolysis, affects the antigen-antibody reaction and a sufficient response cannot be obtained.

The paragraph beginning on page 6, line 4 and ending on line 15, has been amended as follows:

As the erythrocyte lysing agent contained in the aqueous solution used for diluting the resulting agglutination mixture, [are suitably used] suitable agents that can be used are those

capable of not only destroying the membrane of erythrocytes but also dissolving or contracting the membrane. For example, usable are surfactants usually used in the field of counting blood cells for lysing erythrocytes. Particularly, water-soluble surfactants may be mentioned. The water-soluble surfactants may be cationic, anionic, non-ionic or ampholytic. Among these, those having a stronger hydrophobic nature in a hydrophobic part (a larger carbon number) are more preferable because they have a greater ability to lyse erythrocytes.

The paragraph beginning on page 8, line 11 and ending on line 15, has been amended as follows:

A PAMIA series produced by [Sysmex] SYSMEX Corporation provides apparatuses for counting immunoassay. This series is suitable because a single apparatus can perform a set of operations from mixing a sample with a buffer to calculating the degree of agglutination automatically.

The paragraph beginning on page 12, line 19 and ending on line 23, has been amended as follows:

In this Example, RANREAM HBsAg (produced by [Sysmex] SYSMEX Corporation) was used for preparing a sample which was subjected to the latex agglutination and then hemolyzed. PAMIA-30 (produced by Sysmex Corporation) was used for determination.

IN THE CLAIMS

The claims have been amended as follows:

Claim 1 (Amended) A whole blood immunoassay comprising the steps of:

mixing a whole blood sample with sensitized insoluble carrier particles to cause an immune agglutination;

diluting the resulting agglutination mixture with an aqueous solution containing an erythrocyte lysing agent to lyse erythrocytes[, thereby preparing an assay sample]; and

determining a degree of agglutination of the [assay sample] resulting whole blood sample.

Claim 4 (Amended) [An] A whole blood immunoassay according to Claim 1, wherein the degree of agglutination of the assay sample [which] is conducted by [use of an apparatus for a counting immunoassay utilizing a principle of] flow cytometry.

Claim 5 (Amended) A whole blood immunoassay according to Claim 4, further comprising the steps of:

introducing [the assay sample included] the resulting whole blood sample including unagglutinated particles and agglutinated particles to a flow cell, irradiating particles passing through

the flow cell with laser light, and detecting scattered light generated thereby;

setting a threshold value for distinguishing unagglutinated particles from agglutinated particles with regard to intensity of the scattered light; and

distinguishing and counting the unagglutinated particles and the agglutinated particles in reference to the threshold value; and

calculating the degree of agglutination from the number of unagglutinated particles and the number of agglutinated particles.

Claim 11 has been added.